

INCIDENCE OF VARIOUS FUNGAL SPECIES IN OCULAR INFECTIONS

Section: Healthcare Sci. Journal Impact Factor 4.016

Amrita Bajpai¹, Rajesh Bareja², Munesh Sharma³, Vashishth Mishra², Hiba Sami²

Department of Ophthalmology, Rajshree Medical Research Institute, Bareilly, Uttar Pradesh, India; Department of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences, Bhojipura, Bareilly, Uttar Pradesh, India; Department of Microbiology, Rajshree Medical Research Institute, Bareilly, Uttar Pradesh, India.

ABSTRACT

Background: The fungi are significant pathogens causing ocular infections due to their frequent involvement and difficulty in establishing definitive diagnosis. The present study was aimed to detect various fungal and bacterial agents that can cause ocular infections.

Material & Methods: An ophthalmologist collected the corneal scrapping that was smeared onto two slides and stained with Gram's stain and mounted with 10% KOH (potassium hydroxide) for microscopic examination. Material collected was inoculated directly onto 5% sheep's blood agar in the form of 'C' streak, Sabouraud dextrose agar (SDA), and sent to the Microbiology department for further processing and identification of causative agent.

Results: Out of three hundred and sixty samples, ten cases were found to be fungal culture positive. *Aspergillus* species were accounting for 40% cases followed by *Curvularia* species (30%), Alternaria species (1%), *Fusarium* species (1%) and *Scytalidium* dimidiatum (1%).

Conclusion: Fungal infection is a life threatening condition, which needs early diagnosis and treatment to save the patient's eye.

Key Words: Keratitis, Corneal scrapping, KOH, SDA

INTRODUCTION

Leber, in 1879, first time described the fungal infection of the cornea, mycotic keratitis [1]. Since then it has been recognized as a major public health problem in the tropical parts of many developing nations including India [2-4]. The etiological cause for keratitis may vary at different geographical locations [5]. Many fungal genera have been implicated in keratomycosis, like Aspergillus, Fusarium, Curvularia, Alternaria, Penicillium and Bipolaris species. According to World Health Organization (WHO) about 1.5 to 2.0 million new cases of monocular blindness in developing country every year is due to corneal ulceration and corneal ulcer is the second most common cause of blindness after cataract in developing country [6,7]. Fungal infection is a life threatening condition that needs early diagnosis and treatment to save the patient's eye. In view of this context, the present study was conducted to find out various fungal and bacterial agents that can cause ocular infections.

MATERIAL AND METHODS

This prospective study was carried out in a tertiary care hospital during March 2015 to June 2016. The ethical clearance from the ethical committee of the institute was taken to conduct the study. Three hundred and sixty samples of corneal scrapping from clinically suspected cases of corneal ulcer were subjected to bacterial and fungal examination. An ophthalmologist examined all the patients in the eye OPD and ward. A corneal scrape was performed by an ophthalmologist using a sterile 21 gauge needle, or sterile bard parker blade (No.15), following the instillation of 4% lignocaine (lidocaine). Scrapping material was taken from edge and base of ulcer [8]. Corneal material obtained from scraping was smeared onto two slides, which were stained with Gram's stain and mounted with 10% potassium hydroxide (KOH) for microscopic examination respectively. Also material was inoculated directly onto 5% sheep's blood agar (BA) in the form of 'C' streak, Sabouraud dextrose agar (SDA), and sent

Corresponding Author:

Rajesh Bareja, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly, Uttar Pradesh, India - 243202

Ph: +91-9458705236; E-mail: rajeshbareja@gmail.com

Received: 19.09.2016 **Revised:** 01.10.2016 **Accepted:** 13.10.2016

to the Microbiology department for further processing and identification. If there was sufficient specimen of corneal scraping, then it was inoculated on BHI (brain heart infusion) broth and CA (chocolate agar). Inoculated BA, CA plates and BHI broth were incubated at 37°C for 7 days, and discarded after seven days if no growth was observed. Inoculated SDA tubes were incubated at 25°C and 37°C for four weeks. Inoculated tubes were checked once in first week and then twice in every week for maximum period of 3 weeks. Bacteria were further identified using routine biochemical identification tests and selective media [9]. Fungi were identified according to the macroscopic appearance of cultures on SDA and microscopic appearance in LPCB (lactophenol cotton blue) mount [10].

RESULTS

Three hundred and sixty specimens of corneal scrapping from clinically suspected cases of corneal ulcer were subjected to bacterial and fungal examination. Of these, ten cases were found to be fungal culture positive. Among the culture positive specimens, *Aspergillus* species were accounting for 40% cases followed by *Curvularia* species (30%), *Alternaria* species (1%), *Fusarium* species (1%) and *Scytalidium dimidiatum* (1%). All the specimens that were found positive in 10% KOH mount were positive for fungal culture also. None of the bacteria were isolated from bacterial culture.

DISCUSSION

Fungal infections kill at least 1,350,000 patients with or following AIDS, cancer, TB and asthma as well as causing untold misery and blindness to tens of millions more worldwide [11]. Blindness caused by fungal infection of the eye affects over 1 million adults and children globally because the tools are not available for rapid diagnosis and treatment [11]. In 2006, CDC, state and local health departments, and the Food and Drug Administration (FDA) investigated a large, multistate outbreak of *Fusarium* keratitis associated with a specific type of contact lens solution, which was later withdrawn from the market [12-14].

In the present study, *Aspergillus* species were found to be positive in 40% cases. *Aspergillus* species (56.42%) were also repoted the common cause of fungal keratitis by Sherwal et al. [15]. A study from South India also repoted the *Aspergillus* species as the common causitive organism of fungal keratitis [16]. Several other reports from Nepal, Bangladesh and India have also shown *Aspergilus* species as most common isolate in fungal keratitis [17-19]. Another study from North India showed the prevalence of *Aspergillus* species (41%) in fungal keratitis that was concordant to this study [20]. The *Aspergilus* species is most common patho-

gen for fungal keratitis, probably, because it is resistant to hot and dry conditions [5].

In the current study, *Curvularia* species were found to be positive in 30% cases and was the second most common cause of fungal keratits. Some other authors also reported the *Curvularia* species, second most common cause of fungal keratits [15,16]. *Curvularia* species (29%) were second common isolate found in the another study done in North India [20]. Some studies in south India have reported *Fusarium* species to be more common than *Aspergillus* species [16, 21]. In the present study, *Fusarium* species were present in only 1% of the total positive cases. *Fusarium* species have also been found to be the principal fungal pathogen in Florida, Paraguay, Nigeria, Tanzania, Hong Kong, and Singapore [5]. *Aspergillus* species predominate in Northern India, Nepal, and Bangladesh [22-24]. This phenomenon may be explained by differences in climate and the natural environment.

CONCLUSION

The causative fungi of keratomycosis are ubiquitous organisms and are responsible for 6 to 53% of all the corneal infections worldwide [25]. The use of PCR can yield quick results, confirming the diagnosis of mycotic keratitis within a few hours, but in a developing country like India where more than 65% people are from rural area cannot afford the cost of PCR. Direct microscopic examination of KOH mounts appeared as a rapid, reliable, inexpensive and highly sensitive diagnostic method that would facilitate the institution of early antifungal therapy before the culture results became available to limit the ocular morbidity and other complications.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Conflict of interest: Nil

Source of funding: Self financed / Nil

REFERENCES

- Abad JC, Foster CS. Fungal keratitis. Int'l Ophthalmol Clin 1996; 36: 1-15.
- Agarwal V, Biswas J, Madhavan HN, Mangat G, Reddy MK, Saini JS, et al. Current perspectives in infectious keratitis. Indian J. Ophthalmol 1994; 42: 171-2.

- Gunawerdena SA, Ranasinghe KP, Arseceuleratne SN, Scimon CR, Ajello L. Survey of mycotic and bacterial keratitis in Sri Lanka. Mycopathologia 1994; 127: 77-1.
- Hagan M, Wright E, Newman M, Dolin P, Johnson G. Causes of suppurative keratitis in Ghana. Brit J Ophthalmol 1995; 79: 1024-8.
- Leck AK, Thomas PA, Hagan M, Kaliamurthy J, Ackuaku E, John M, et al. Aetiology of suppurative corneal ulcers in Ghana and south-India, and epidemiology of fungal keratitis. Br J Opthalm 2002; 86: 1211-5
- Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ vol 2001; 79(3): 214-1.
- Nayak N. Fungal infections of the eye laboratory diagnosis and treatment. Nepal Med Coll J 2008; 10(1): 48-3.
- 8. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, et al. Epidemiology and etiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalm 1997; 81: 965-1.
- Collee, JG, Fraser AG, Marmion BP, Simmons A. Tests for the identification of Bacteria. Mackey and McCartney practical Medical Microbiology, New Delhi, India: Elsevier 2006; 131-9.
- Fisher F, Cook NB. Fundamentals of diagnostic mycology. Saunders, Philadelphia, PA. 1998; 13-4.
- www.gaffi.org/global-plague-how-150-people-die-every-hourfrom-fungal-infection
- Chang DC, Grant GB, O'Donnell K, Wannemuehler KA, Noble-Wang J, Rao CY, et al. Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. JAMA 2006; 296: 953-3.
- CDC. Update: Fusarium keratitis—United States, 2005-2006. MMWR 2006; 55: 563-4.
- CDC. Fusarium keratitis—multiple states, 2006. MMWR 2006;
 55: 400-1.

- Sherwal BL, Verma AK. Epidemiology of ocular infection due to bacteria and fungus – a prospective study. J Med Edu Res 2008:10(3); 127-1.
- Kuniomoto DY, Sharma S, Garg P, Gopinathan U, Miller D, Rao GN. Corneal ulceration in the elderly in Hyderabad, south India. Br J Ophthalmol 2000; 84: 54-9.
- Yeh DL, Stinnett SS, Afshari NA. Analysis of Bacterial culture in infectious kerititis 1997-2000. Am J Opthalmol 2006; 142; 1066-8.
- 18. Dunlop AA, Wright ED, Holader SA, Nazrul I, Jussain R, Mc-Clellan K, et al. Suppurative corneal ulceration in Bangladsh. A study of 142 cases examining the microbiological diagnoses, clinical and epidermological feature of bacterial and fungal keratitis. Aust NA J Opthalmol 1994; 22: 105-10.
- 19. Mahajan VM. Ulcerative keratitis: An analysis of laboratory data in 674 cases. J Ocul Ther Surg 1985; 4: 138-1.
- Chowdhary A, Singh K. Spectrum of Fungal Keratitis in North India. Cornea 2005: 24(1); 8-15
- Gopinathan U, Garg P, Merle F, Sharma S, Sreedharan A, Rao GN. The Epidemiological Features and Laboratory Results of Fungal Keratitis: A 10-Year Review at a Referral Eye Care Center in South India. Cornea 2002: 21(6); 555-9.
- 22. Chander J, Sharma A. Prevalence of fungal corneal ulcers in northern India. Infection 1994; 22: 207–9.
- Upadhyay MP, Karmacharya PC, Koirala S, Tuladhar NR, Bryan LE, Smolin G, et al. Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991; 111: 92-9.
- Williams G, McClellan K, Billson F. Suppurative keratitis in rural Bangladesh: the value of gram stain in planning management. Int Ophthalmol 1991; 15:131–5.
- Thomas P. Mycotic keratitis an underestimated mycosis. J Med Vet Mycol 1994; 32: 235–6.